Section - 5

Results and Discussion

5. RESULTS AND DISCUSSION

This section has been divided into four major parts. Apart from describing the results, we have compared and evaluated our findings with the recently available findings in the discussion part. Also the analysis of the demographic characteristics of the population included in each of the study had been done. This has also been evaluated in the discussion part. Furthermore, a comprehensive analysis of the demographic characteristics of the individuals included in the study have been done and correlated with the available hypothesis and findings in schizophrenia.

5.1 HLA CLASS I TYPING

5.1.1 RESULTS

The incidence and frequency of HLA Class I genes among patients and controls have been presented in table 15 and 16. As the patient groups consist of mostly paranoid subtypes and the number of patients of the different subtypes were few (Table 25), we have considered the schizophrenic patients as a whole. The result of the HLA study showed a significantly higher frequency of HLA-A*03 (χ^2=77.519, p<0.001) in patients than the control groups. On the other hand A*31 (χ^2=34.160, p<0.001) and B*51 (χ^2=31.083, p<0.001) showed significantly lower value even after the Bonferroni corrections (Fig.23).

Table 15: Allele frequency, Chi square, relative risk (RR) values and probability of HLA-A loci alleles in the patients with schizophrenia and healthy controls.

	Patients (N=136)	Control (N=150)			
Allele	Allele Frequency %	Allele Frequency %	Chi square	RR	P value
A1	11.8	12.3	0.050	0.941	0.466
A2	15.4	17	0.316	0.869	0.332
A3	39.7	13.7	77.519	10.045	<0.001 *
A11	18	18	0.000	1.002	0.449
A23	7.7	6.3	0.456	1.255	0.434
A24	11.8	14.3	0.973	0.769	0.197
A25	10.3	15	3.324	0.609	0.045
A26	11.4	15	1.898	0.692	0.107
A29	12.1	8.3	2.547	1.593	0.271
A30	12.1	10	0.755	1.279	0.438
A31	0.7	13.3	34.160	0.051	<0.001 *

* significant after the Bonferroni's correction, Bonferroni's probability is 0.0025

Table 16: Allele frequency, Chi square, relative risk (RR) values and probability of HLA- B loci alleles in the patients with schizophrenia and healthy controls.

	Patients (N=136)	Control (N=150)			
Allele	**Allele Frequency %**	**Allele Frequency %**	**Chi square**	**RR**	**P value**
B7	17.3	13	2.485	1.498	0.357
B8	15.8	16.7	0.096	0.926	0.428
B18	9.2	11.3	0.799	0.772	0.228
B37	11.4	13.3	0.573	0.815	0.268
B40	6.9	8.3	0.398	0.817	0.321
B42	10.7	9	0.500	1.232	0.397
B44	4.4	2.7	1.336	1.683	0.321
B49	8.5	5.7	1.845	1.580	0.303
B51	0.5	11.7	31.083	0.036	<0.001*

*significant after the Bonferroni's correction, Bonferroni's probability is 0.0025

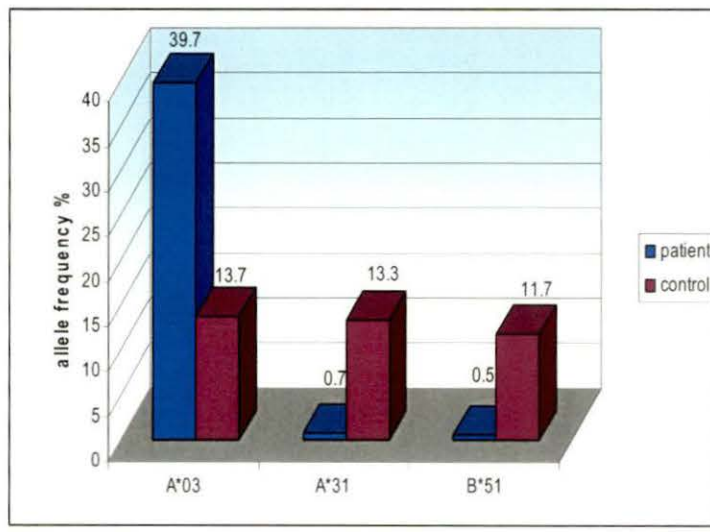

Figure 23: Bar diagram showing the frequency of some of the alleles in the patients and controls that showed the association with schizophrenia.

Significant HLA-A and B haplotypes among the schizophrenic patients and healthy control subjects are presented in the table 17 and 18. Some of the prominent haplotypes observed in the patients were not seen amongst the healthy subjects. The haplotype A1-B8 (χ^2 = 12.216) and A23-B49 (χ^2 = 11.444) were found to have the higher χ^2 value among the patient group, whereas among the control A26-B51 (χ^2 = 8.365) A01-B37(χ^2 =6.861) were found to have the higher χ^2 value.

Table 17: Significant haplotypes and delta values per 1000 among schizophrenic patients.

Haplotype	HF	Delta	χ^2
A1-B8	126.448	71.864	12.216***
A11-B8	163.800	73.719	8.616**
A11-B49	95.555	49.789	6.749**
A23-B49	58.634	42.041	11.444***
A24-B40	63.539	42.203	9.242**
A26-B49	57.987	40.505	10.101**

* = significant
Note: *= p<0.05, **=p<0.01, ***=p<0.001
HF=Haplotype frequency per 1000.

Table 18: Significant haplotypes and delta values per 1000 in normal individuals.

Haplotype	HF	Delta	χ^2
A01-B37	499.343	96.328	6.861**
A01-B40	346.586	75.501	5.270*
A25-B51	133.304	55.997	6.090*
A26-B51	184.790	73.245	8.365**
A29-B40	366.394	77.904	4.237*
A30-B51	179.267	58.369	4.904*
A31-B40	317.260	81.835	6.614*

* = significant
Note: *= p<0.05, **=p<0.01, ***=p<0.001
HF=Haplotype frequency per 1000.

5.1.2 DISCUSSION

Several researchers have the opinion that the likely schizophrenia locus is on chromosome 6p close to the human leukocyte antigen (HLA) region by linkage analysis (Moises *et al.*, 1995; Schwab, 1995; Wang, 1995; Straub, 1995; Antonarakis *et al.*, 1995; Schizophrenia Linkage Collaborative Group, 1996; Levinson *et al.*, 1996; Schwab *et al.*, 1998; Lindholm *et al.*, 1999; Li *et al.*, 2001; Schwab *et al.*, 2002). Since the first study done by Cazzolo *et al.*, 1974 , more than 80 schizophrenia HLA

association studies have been reported till to date (Bogacki, 2005). A recent study also found the significant increase in the frequency of a SNP in HLA-DOA in schizophrenia and a significant decrease in the frequency of a SNP in HLA-DRB1 region (Herbon, 2003). Furthermore genome-wide association study also found the association of schizophrenia in chromosome 6p (The International Schizophrenia Consortium, 2009).

The major findings of the present study is the increased frequency of HLA-A*03 and decreased frequency of HLA A*31 and B*51 among the schizophrenic patients. Thus, our results also strengthen the possibility of likely schizophrenia locus on chromosome 6p close to HLA region. A significant higher frequency of HLA-A*03 observed in the present study is in accordance with the previous study reported by Debnath *et al.,* (2005) but unlike the present study only paranoid schzophrenics were considered for the study. Moreover their sample size consisted of only fifty patients. The result of the present study is also in accordance with the study of Rudduck *et al.*, (1984) in Swedish population. On the other hand the lower frequency of HLA A*31 and B*51 observed in the patient group is the new findings of the present study than the previous study. Moreover the frequency of HLA-A*25 was also found to be lower among the patient groups but were not statistically significant. The increased frequency of A*11 found in the previous study by Debnath *et al.*, (2005) was not reproducible in the present study. However we have not found any association between HLA-A*23 and A*24, the most consistent association reported so far in schizophrenia (Ivanyi *et al.*,1983; Asaka *et al.*, 1981).

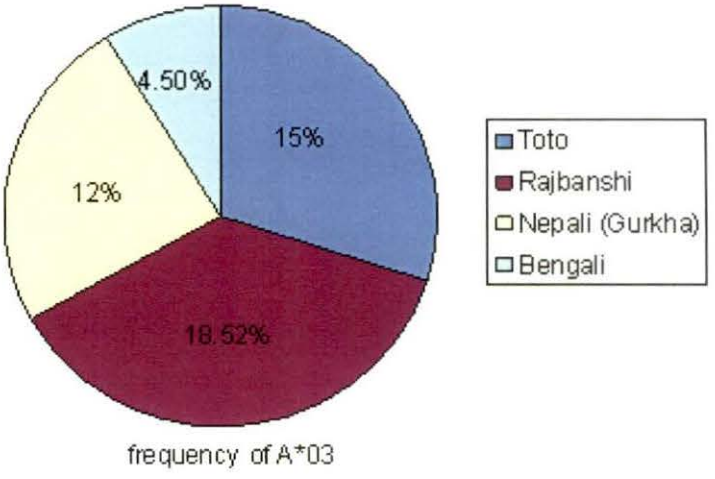

Figure 24: Frequency of HLA A*03 alleles in some of the population of North Bengal.

It is also to be noted that utmost care have been taken to match the case and control subjects in this study and our results could not be an artifact arising from the inadvertent ethnic

mismatching of cases and controls, as there is no ethnic group known in this region for which the HLA-A*03 frequency is higher than about 19%. The pattern of HLA-A*03 distribution in some of the population of India, especially in West Bengal is as follows: Indian tribe 6.0%(Imanishi *et al.*, 1992), Rajbanshi population 18.52% (Mandal *et al.*, 2000), Nepali (Gurkha)12%(Chaudhuri *et al.*, 1995) and Bengali 4.5% (Singh *et al.*, 2009) (Fig. 24).

Significant HLA-A and B haplotype among the schizophrenic patients and healthy control subjects are shown in the Table 3 and 4. The most significant haplotype HLA A1-B8 observed among the patient is the most common Caucasian haplotype, associated with the several autoimmune diseases such as type 1 diabetes (Degli-Esposti *et al.*, 1992; Cheong *et al.*, 2001) celiac disease (Sollid and Thorsby, 1993), common variable immunodeficiency (Schroeder *et al.*, 1998) , myasthenia gravis (Vandiedonck *et al.*, 2004) and systemic lupus erythematosus (Christiansen *et al..*, 1991). Thus our results hint towards the autoimmunological background of schizophrenia.

The exact nature of the mechanism underlying the empirically observed association of the HLA-A*03 gene with schizophrenia remains unknown. However, several genetic and environmental factors may be involved with such an association. HLA generally plays a critical role in the control of infectious and other immune functions. Many microbial factors have been implicated in the pathogenesis of schizophrenia, but so far each microbial factor has been identified in a relatively small subgroup of patients (Bechter, 1998; Karlsson, 2001). The heterogeneity of these microbial factors are also reflected by the associations with different HLA loci and their alleles. The set of inherited HLA alleles determine the susceptibility or resistance to particular microbes (Laumbacher, 2003). Also, it is widely accepted that a disturbance in neurodevelopment may be related to the development of schizophrenia. Results have also shown that an interaction between HLA and a perinatal or prenatal infection, which can affect neurodevelopment, may be associated with schizophrenia (Narita, 2000).

The analysis of the demographic data suggests that the present study comprises more number of paranoid schizophrenic patients (Table 25). On the other hand the present schizophrenic population comprises more number of Bengali populations. However, as mentioned earlier they were strictly matched according to their ethnicity, age and sex with the controls. The study comprised more number of male schizophrenic patients and duration of illness was found to be longer in them. On the other hand the study comprises more number of married schizophrenic patients.

The present study further strengthens the earlier findings regarding the association of HLA-A*03 with schizophrenia along with the negative association of HLA A*31 and B*51. Nevertheless, it is too early to speculate the exact mechanisms of the association. However, the study suggests that an immunological mechanism may contribute in the etiology of schizophrenia. In the present study the sample size of the different subtypes of schizophrenic patients were small for detecting the real differences in the HLA distribution among them. This is the limitation of the present study. Taking this into account, the present study suggests the possible existence of a susceptible locus for schizophrenia within the HLA region.

5.2 INTERLEUKIN ASSAY

5.2.1 RESULTS

The results of the IL-2 and IL-6 assay are summarized in table 19 and 20 and figure 25 and 26 and the absolute value of IL-2 and IL-6 are plotted in the dot scatter diagram in figure 27 and 28 respectively. The serum IL-2 level in antipsychotic medicating patients were found to be significantly lower (34.54±22.09 pg/ml) than the control subjects (56.04±18.82 pg/ml) ($p<0.001$). The serum IL-2 level in psychotropic medication free patients (38.76±27.23 pg/ml) were also found to be significantly lower than the normal controls (56.04±18.82 pg/ml) ($p<0.05$).

Table 19: Comparison of serum IL-2 levels between psychotropic medication free, antipsychotic medicating schizophrenic patients with the normal controls.

Variables	Psychotropic medication free schizophrenic group (n=20)	Control (n=30)	P value	
IL-2 (pg/ml)	Mean= 38.76 SD=27.23	Mean=56.04 SD=18.82	P<0.05 *	t= - 2.656 d.f.=48

Variables	Antipsychotic medicating schizophrenic group (n=30)	Control (n=30)	P value	
IL-2 (pg/ml)	Mean=34.54 SD=22.09	Mean=56.04 SD=18.82	P<0.001*	t= - 4.058 d.f.=58

* = significant

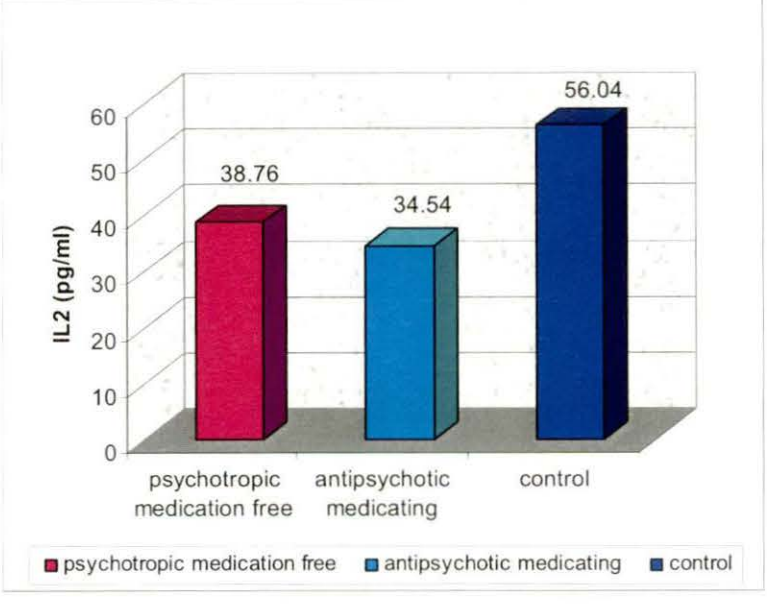

Figure 25: Comparison of serum level of IL-2 between psychotropic medication free, antipsychotic medicating patients and normal controls.

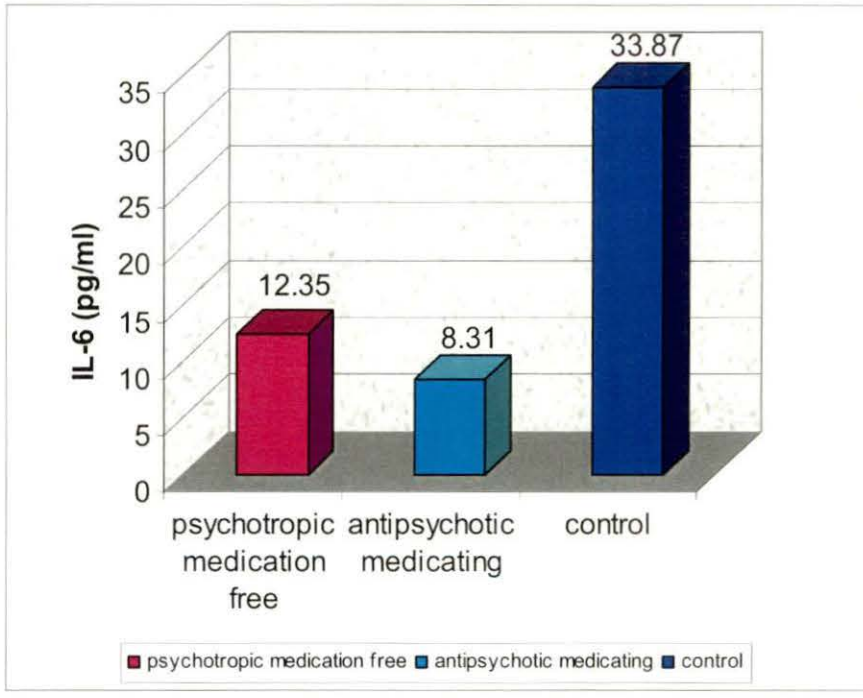

Figure 26: Comparison of serum level of IL-6 between psychotropic medication free, antipsychotic medicating patients and normal controls.

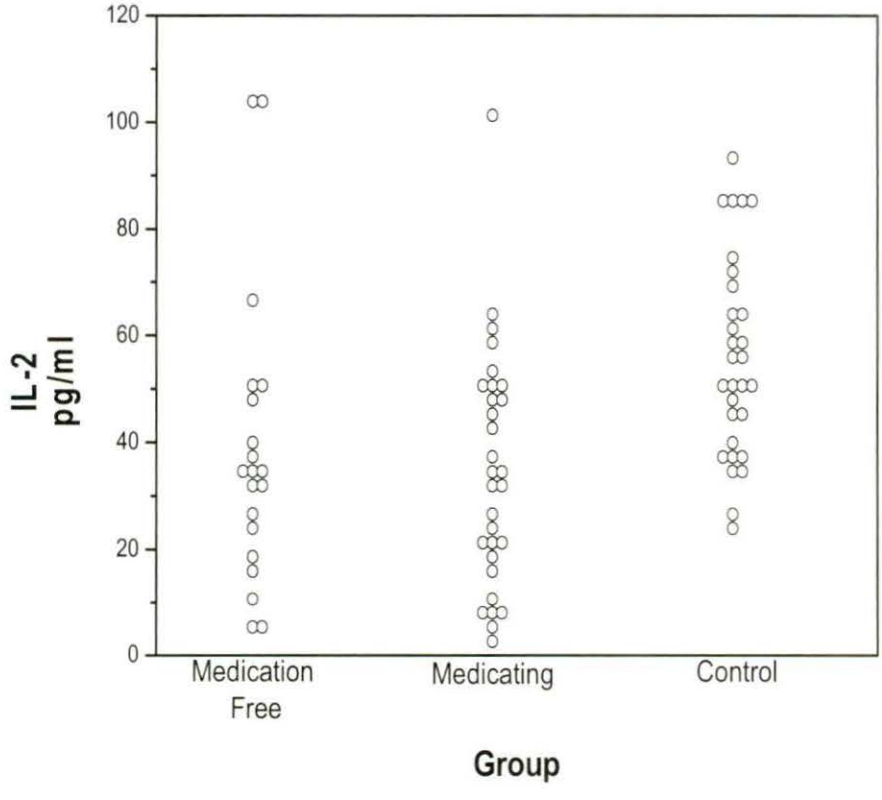

Figure 27: Dot scatter diagram of serum IL-2 level (pg/ml) between medication free, medicating schizophrenic patients and control.

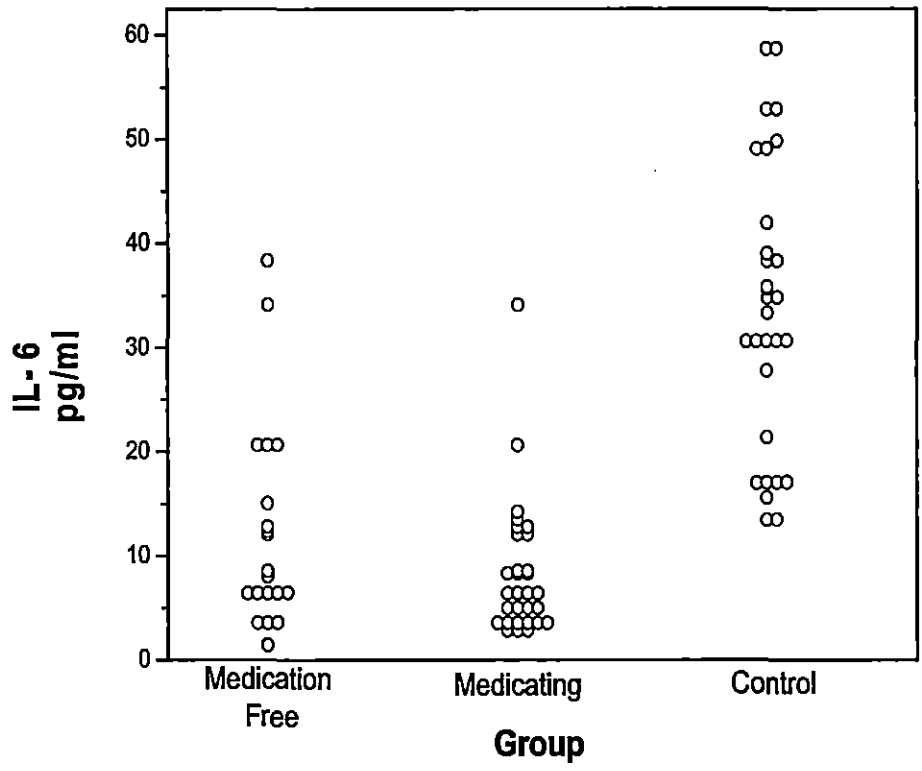

Figure 28: Dot scatter diagram of serum IL-6 level (pg/ml) between medication free, medicating schizophrenic patients and control.

Table 21: BPRS score between medication free and medicating schizophrenic patients.

Medication free BPRS (N=53)	Mean=42 SD=2.402	P<0.001***	t=5.505 d.f.=134
Medicating BPRS (N=83)	Mean=40 SD=1.821		

*=p<0.05, **=p<0.01,***p=<0.001
* = significant

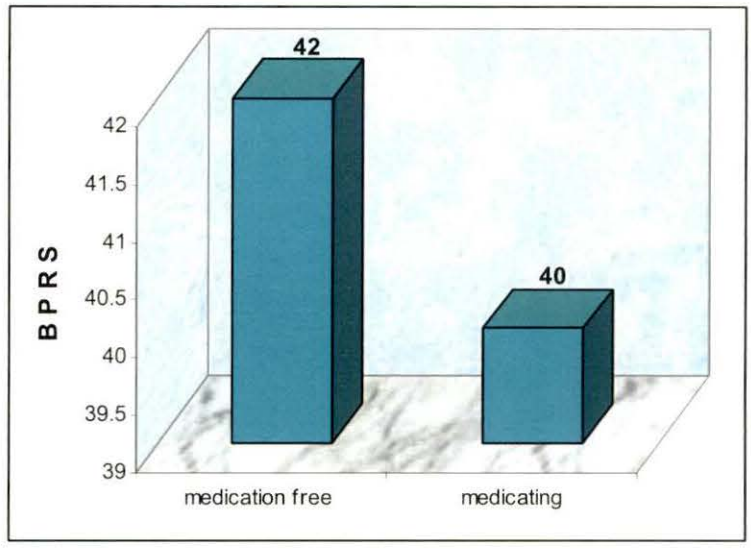

Figure 29: BPRS score depicted in the bar diagram in medication free and medicating patients.

Table 22: Demographic characteristics of the schizophrenic patients and the control subjects.

	Schizophrenia N=50	**Mean**	**standard deviation (SD)**		**Control N=30**	**Mean**	**standard deviation (SD)**
Sex, Male Female	 30 (60%) 20 (40%)				 18 (60%) 12 (40%)		
Age		32.36	10.50			33.87	11.35
Duration of illness		5.57	5.47				
Duration of treatment in years (N=30)		3.33	4.19				
Age of onset		26.18	9.58				
Subtypes of schizophrenia Paranoid Residual	 44 (88%) 1 (2%)			χ^2 =106.32			

	Schizophrenia N=50	Mean	standard deviation (SD)		Control N=30	Mean	standard deviation (SD)
Disorganized Catatonic	4 (8%) 1 (2%)			(d.f.=3) p<0.001 *			
Medication status of patients Psychotropic medication free Antipsychotic medicating	20 (40%) 30 (60%)			χ^2=2.00 (d.f. =1)			
Body mass index (kg/m^2)		22.496	1.426			22.657	1.514 df=78 P<0.635
Smoking status	0				0		
Antipsychotic types and dose Olanzapine (10-20 mg/day) Quetiapine (300mg-600mg/day) Clozapine (100mg-300mg/day) Risperidone (2mg-8mg/day)	(N=30) 8 8 4 10						
BPRS score Psychotropic medication free Antipsychotic medicating		42 40	2.714 1.702	t = 3.207 df = 48 p<0.01 *			

Abbreviation: IL= Interleukin, BPRS=Brief psychiatric rating scale

* = significant

5.2.2 DISCUSSION

To the best of our knowledge this is the first attempt to study the role of interleukins in the Indian schizophrenic subjects. The lower level of IL-2, observed among the schizophrenic patients in the present study is in agreement to Theodoropoulou *et al.*, (2001). However our findings are in contrast to the previous findings of Ebrinc *et al.*, 2002 and Zhang *et al.*, (2002, 2005) who have found elevated level of IL-2 in their subjects. Also this finding is in contrast to the studies of Kim *et al.*, (1998, 2000) who found an increase of serum IL-2 level in Korean schizophrenic patients.

The unique finding of the present study is the significantly lower level of IL-6 in the schizophrenic subjects. To our knowledge this is the first report of lower level of IL-6 in the schizophrenic patients and nowhere else have been reported previously. The finding is in contrast as reported by Zhang et al., 2002, who have found the elevated level of IL-6 in the schizophrenic group than the normal control. On the other hand some other groups have reported no significant differences between the patients and the controls (Baker *et al.*,1996).

The non agreement of some of the previous and present findings may be due to the different assay methods, differences in test materials (serum vs plasma), sampling of patients in different stages of disease progression (acute vs chronic or active phase vs remission), exposure to a variety and duration of neuroleptic treatments and different disease progressions. Much of the current research works are directed towards the heterogeneity of schizophrenia (Graver *et al.*, 2003). Pulver (2000) makes this point by describing schizophrenia as a syndrome with 'genetic heterogeneity' having susceptibility loci at several different chromosomal regions. Kirkpatrick and Carpenter have proposed strong evidence in support of a dichotomy, 'deficit' and 'nondeficit' schizophrenia (Kirkpatrick *et al.*, 2001). Garver *et al.*, (1988) also delineated and subsequently replicated three distinct clusters or 'endophenotypes' within the group of patients that meet conventional criteria for the DSM-IV schizophrenia syndrome (Garver *et al.*, 1999; Garver *et al.*, 2000). If such etiologically distinct endophenotypes exist, it should be suspected that central immune activation may be a component of one, but not all of the endophenotypes. On the other hand statistically significant ethnicity-based variability in the allelic

frequency and genotype inheritance patterns for IL-2 and IL-6 has been reported (Hoffmann *et al.*, 2002) suggesting the polymorphisms within these cytokine genes may be responsible for the ethnic-based differences in IL-2 or IL-6 levels (Zhang *et al.*, 2005). This may be one of the reasons for the difference in the opinions regarding the results.

The immunosuppressive and cytokine modulating effects of the antipsychotic drugs have been found by different studies (Maes *et al.*, 1995; Song *et al.*, 2000). Atypical antipsychotics such as risperidone and clozapine also appear to have anti-inflammatory activities (Song *et al.*, 2000; Leykin *et al.*, 1997; Maes *et al.*, 2000). In the present study only atypical antipsychotics were prescribed to the patients (Table 22). Therefore the lower levels of IL-2 and IL-6 observed among the medicating patients in the present study suggest the cytokine modulating activity of the atypical antipsychotics. Thus, our findings support the earlier findings that treatment with antipsychotic drugs affect the cytokine network (Pollmacher *et al.*,1996; Schuld *et al.*, 2004).

The results of our findings do not agree with the exhaustion theory of schizophrenia which is characterized by decreased production of IL-2 *in vitro* and increase of IL-2 in serum (Ganguli *et al.*,1989; Ganguli *et al.*,1995; Villemain *et al.*, 1989; Arolt *et al.*, 2000) and increase in IL-6 *in vitro* (Maes *et al.*,1995; Muller *et al.*, 1997, Van Kammen *et al.*, 1999). Also, our findings do not fit well into the Th1/Th2 paradigm or with the Th2 shift hypothesis. Until recently, IL-2 and IL-6 were classified as Th1 and Th2 type cytokines respectively (Mosmann *et al.*, 1986; Romagnani, 1995; Lucy *et al.*, 1996).This probably contributed to the formulation of the hypothesis of a shift from Th1 to Th2 cytokines on the basis of studies showing decreased *in vitro* IL-2 secretion and increased *in vivo* circulating sIL-2R and IL-6 levels in schizophrenia (Schwarz *et al.*, 2001). However, classification of cytokines is being re-examined, because new CD4+ T cell subsets, such as Th17 and Treg (Dong, 2006 ;Tato *et al.*, 2006) are emerging. The data on Th17 and Treg defining cytokines in schizophrenia are still lacking or insufficient at this point of time because of the lack of novelty of the findings and awaits further research (Potvin *et al.*, 2008).

Table 22 shows the demographic characteristics of the patients and normal controls. No significant relationships between age, sex, BMI and serum IL-2 and IL-6 were observed. Age of onset and duration of illness did not significantly correlate with the IL-2 and IL-6 levels in the patient groups. The patient group consists mostly of Paranoid schizophrenics (88%).

The limitations of the present study include the successive follow-up, which could not be done on the same patients to understand the effect of antipsychotics on the serum level of interleukins. This is because the same patients did not turn up in the OPD to follow up the treatment. However, it is evident from our study that antipsychotics downregulate the serum level of interleukin.

To conclude, the results of our present findings strengthens the earlier findings of the immune system dysregulation in schizophrenia which may be one of the etiological factors for the disorder. However our finding does not fit well to the autoimmune hypothesis of schizophrenia. Further studies involving other cytokines and *in vitro* cytokine production may throw light in this respect. Additional studies are invited further to unfold the effect of antipsychotics in the immune system which may help in future drug development.

5.3 C-REACTIVE PROTEIN TEST

5.3.1 RESULTS

Sera levels of CRP were measured for 64 schizophrenic patients. Among them the elevated level of CRP (≥6 mg/L) was observed in 3 patients and 61 patients were found to have the normal CRP (<6mg/L). All the three elevated cases were found to be of paranoid type. No differences were found in CRP levels among the different subgroups of schizophrenia. Further, when the level of CRP was compared to the other demographic variables as shown in table 23, only the drug naïve status of the patients showed statistically significant value ($\chi^2 = 16.997$, p value$<3.75 \times 10^5$).

Table 23: Comparison of demographic and clinical characteristics between the normal / elevated CRP groups.

	Elevated CRP N=3		Normal CRP N=61			
	Mean or N%	Standard deviation	Mean or N%	Standard deviation	Statistic (Z)	*P* value
Age	37.67	21.13	34.69	9.64	F[2,60]=0.24	>0.62
Gender Men Women	33.33%		70.49%		X^2 [1]=1.84	>0.17
Drug naïve patients Patients under antipsychotic medication	100%		11.48%		X^2 [1]=17.00	<0.001 Significant
Substance abuse Yes No	33.33%		55.74%		X^2[1]=0.58	>0.44
First child Yes No	33%		18.03%		X^2[1]=0.44	>0.50
Autoimmune disease in patients or in family Yes No	0%		24.59%		X^2[1]=0.97	>0.32

5.3.2 DISCUSSION

This preliminary first hand study provides further evidence of the involvement of inflammatory processes behind the etiopathology of schizophrenia. The elevated level of CRP in our study is in accordance to the findings of Fan *et al.*, 2007 and Dikerson *et al.*, 2008. But unlike previously reported findings, we have considered the CRP level of patients with their medication status, which showed significantly higher value. In the study by Fan *et al.*, 2007 the level of CRP was found to be higher in the patiens, who were experiencing psychotic symptoms. In the follow up study of non-psychotic state, the level of CRP was found to be normal. In this respect, the present study suggests that the antipsychotic drug may perhaps down regulate the inflammatory process, which in turn brings the CRP level to the normal state.

Thus, these findings further suggest that the inflammation may be another possible mechanism in the etiopathology of schizophrenia. It is, however, not clearly understood whether the elevation of the level of CRP is the by-product of the pathophysiology of schizophrenia or directly contributes to the clinical manifestation of the disorder (Fan *et al.*, 2007).

Until now, it is not clearly understood regarding the mechanism of inflammation in schizophrenia. It is suggested that the vascular-structural brain abnormalities may be one of the factors in the etiology of schizophrenia like psychoses (Howard *et al.*, 2001; Bachneff, 1996; Shinba, 2004). It is proposed that chronic inflammation might damage the micro-vascular system in the brain and cerebral blood flow (Hanson, 2005). Further, scientific evidence suggests that an increase in the stress hormone like norepinephrine may activate the inflammatory arm of the immune system and triggers the expression of genes that cause chronic, low-grade inflammation. This inflammation is characterized by the degree of the levels of CRP (Boyle *et al.*. 2007).

This is possibly the first reported study of the association between CRP and the schizophrenia in the Indian scenario. The psychopathology measures of the patients were not considered unlike the previous studies. This is the limitations of the present study. In contrary to the studies conducted by Fan and Dickerson, the higher cut off value (6mg/L) was used for the CRP levels. The sample size of the present study was small. The patients were recruited from the OPD which has limited the follow-up study. Nevertheless, taking this limitation in account the present study provides further evidence that some kind of inflammation may play a role in the etiopathology of schizophrenia. The study further reveals the immunomodulatory effect of the antipsychotic drugs in the patients.

5.4 CD4+ AND CD8+ COUNT

5.4.1 RESULT

The results of the CD4+ and CD8+ cell count have been presented in table 24 and summarized in figure 30. Although the mean percentage of CD4+ cells was found to be little higher (36.10± 4.59) in the patients. It was not significantly higher than the control groups (34.50±6.62). Also the mean percentage of CD8+ cells is not found to

be significantly deviated in patients (29.7 ± 6.72) from the control (28.1±6.38) groups. On the other hand the CD4+ and CD8+ subset ratio did not show any significant deviation between the patients (1.34 vs 1.29) and the control groups. Further, there was no significant correlation between the percentage of CD4+ cells and the serum IL-2 level.

Table 24: The result of the CD4+ and CD8+ count.

	CD4%		CD8%		CD4-CD8 ratio	
	Patient (N=20)	Control (N=20)	Patient (N=20)	Control (N=20)	Patient (N=20)	Control (N=20)
Mean	36.1	34.5	29.7	28.1	1.34	1.29
SD	4.59	6.62	6.72	6.38	0.46	0.39
t-value	- 0.887 df=38		-0.772 df=38		-0.427 df=38	
p-value	0.381		0.445		0.67	
Mann Whitney U test	171.00		163.5		190.5	
P value	0.432		0.322		0.797	

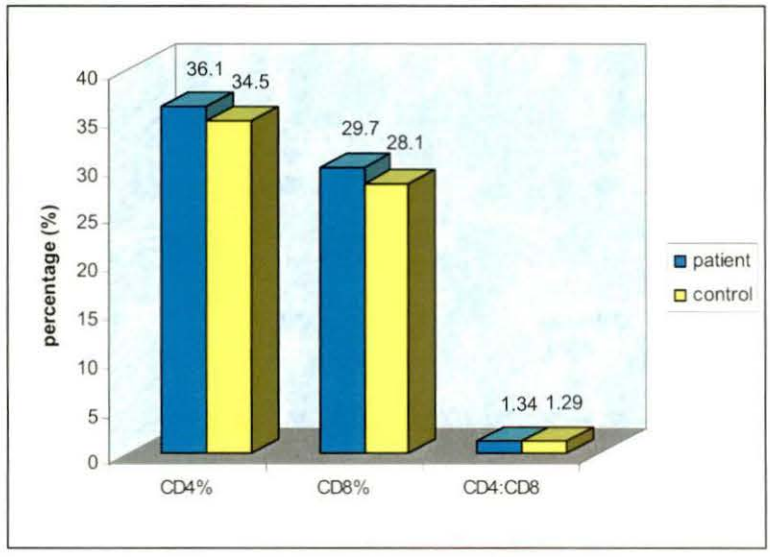

Figure 30: Showing bar diagram of CD4+ and CD8+ cell percentage and CD4-CD8 ratio in patients and controls.

5.4.2 DISCUSSION

The results of our findings regarding the CD4+ cells reflected the findings of Villemain *et al.*, (1989), Achirion *et al.*, (1994) and Baskak *et al.*, (2008), who also did not find any difference among CD4+ cells. However our finding is in contrast to the findings of some other workers who have reported decreased number of CD4+ cells (Zhang *et al.*, 2002; Cosentino *et al.*, 1996). Moreover, some workers have also reported the increased number of CD4+ cells (Ganguli *et al.*, 1987; Henneberg *et al.*, 1990; Muller *et al.*, 1993; Cazzullo *et al.*, 1998a; Sperner-Unterweger *et al.*, 1999). On the other hand our result on CD8+ cells are in agreement with the Sperner-Unterweger *et al.*, (1999) who also found no significant difference, but not in agreement with the Cazzullo *et al.*, (1998a) who have found increase of CD8+ cells. Villemein *et al.*, 1989 on the other hand found significant decrease in CD8+ cells in schizophrenic patients which is in agreement to the work of Achiron *et al.*, 1994 and Baskak *et al.*, (2008). Our findings on CD4+ and CD8+ ratio is not in agreement to the findings of Sperner-Unterweger *et al.*, (1999) who have found higher CD4/CD8 ratio than healthy controls.

For CD4 T cells, the Th1 and Th2 T cells are categorized by the cytokines that they produce. IL-2 is released into the circulation at high levels from CD4+ (Th1) cells upon activation. One of the most frequently reported immunological alterations in schizophrenia is an imbalance in T helper 1 (Th1) and T helper 2(Th2) cytokine profile production with increase Th2 cytokine secretion *in vivo* and decrease *in vitro* (Muller *et al.*, 1999; Muller *et al.*, 2000), suggest diminished pro-inflammatory Th1 responses in schizophrenia. Lower number of CD4+ cells and hence IL-2 production have also been reported in schizophrenia (Zhang *et al.*, 2002), although Muller described the increased CD4+ cell number (Muller *et al.*, 1991) in addition to the altered plasma and serum levels of other cytokines such as IL6 (Frommberger *et al.*, 1997). With respect to the above mentioned studies, our findings are of complete contrast i.e., low level of IL-2 but normal percentage of CD4 cells. This suggests that the abnormal production of ILs is not due to the abnormal number of CD4 cells but it may be due to some abnormalities in the CD4 cells. However given the size of our sample the results should be interpreted cautiously. It is a well known fact that IL- 2 is not solely secreted by the CD4 cells, hence there can be other factors for this

contrasting results. Moreover the knowledge about the new CD4 T cell subsets such as Th17 and Treg are emerging. It is now evident that the Treg cells are capable of suppressing the function of the other T cells. Treg cells have been shown to play a role in regulation a variety of immune responses from autoimmunity to viral infection (Levings *et al.*, 2006).The exact mechanism about these cells triggers autoimmunity has not been revealed yet (Miyara and Sakaguchi, 2007). As the knowledge about these cells will grow, the role of CD4+ and CD8+ T cells in the etiopathology of schizophrenia will become understandable.

5.5 DEMOGRAPHIC STUDIES

5.5.1 RESULT AND DISCUSSION

The comprehensive demographic data of all the patients and controls included in this study have been presented in table 25 which shows the study comprises more number of paranoid (118) schizophrenic patients. On the other hand, most of the patients included in the study belong to Bengalee community (63.97%). However, utmost care was taken to strictly match the patients and controls according to their ethnicity, age and sex. The present study comprises of more number of male schizophrenics and they have long duration of illness compared to females. The data hints the higher vulnerability of men to schizophrenia at least in this region. A comprehensive study involving large number of samples should be done in order to shed light in this respect. The higher number of married patients in this study hints the strict social customs and strong social bondage of the Indian society but again an elaborate study awaits before making any conclusion in this respect. Although it has been hypothesized that the onset of schizophrenia is earlier in males but our result did not show any significant difference in the age of onset between male and females. When the family history of the psychiatric and autoimmune disorder was analyzed we did not found any association, rather we found the significantly higher number of patients who did not have family history of psychiatric and autoimmune disorders. Regarding the educational status it has been noticed that most of the patients did not continue their study beyond class X and there were more number of patients whose educational status was less than VIIIth standard. On the other hand the study also comprises a good number of illiterate patients. Studies have found the increase incidence of

smoking among schizophrenics, but this finding was not reproducible in the present study. One of the interesting observations of the present study is the significant association of schizophrenia with the patients who are not the first child. Our finding is in accordance to the study of Sham *et al.*, (1993) which suggests that in addition to the genetic predisposition, some environmental factors such as viral infection might play a pivotal role on the onset of the disorder and the first child may be the source of infection. Thus the present study strengthens the hypothesis of "younger children in a family has a significantly increased risk of later developing schizophrenia."

Table 25: Comprehensive demographic characteristics of the schizophrenic patients and controls.

	Patients N=136			**Control N=150**		
Gender						
Male	85			92	X^2=0.041	
Female	51			58		
Ethnicity						
Bengali	87	X^2=165.618				
Nepali	9	P<0.001 ***				
Bihari	11					
Tribal	17					
Rajbanshi	12					
Subtypes						
Paranoid	118					
Disorganised	9	X^2= 380.176				
Catatonic	1	P<0.001				
Undifferentiated	5					
Resudal	3					
Marital status						
Married	79	X^2= 66.211				
Unmarried	54	P<0.001***				
Left/divorcee	3					
Age						
Male (N=85)		Mean=33.788 SD= 10.638		Male (N=92)	Mean=33.467 SD=10.524	t=0.2016 d.f.=175
Female(51)		Mean=32.098 SD=10.092		Female (N=58)	Mean=32.190 SD=8.914	t=-0.050 d.f.=107

	Patients N=136			Control N=150		
Duration of illness						
Male (N=85)		Mean=6.827 SD=6.659	t=2.333 d.f.=134			
Female (N=51)		Mean =4.386 SD=4.358	p=0.021 p<0.05*			
Age of onset						
Male (N=85)		Mean=26.145 SD=9.579	t= – 1.054 d.f.=134			
Female (N=51)		Mean=27.944 SD=9.626				
Education						
Nil	24	X^2_5=51.112				
<class VIII	46	P<0.001***				
<Class X	33					
Class X pass	19					
Higher secondary	8					
Graduation	6					
Family history of:-						
Psychiatric disorder	17	X^2_2=134.610 P<0.001***				
Autoimmune disorder	10					
No family history	109					
Substance abuse						
Smoking	37	X^2_2=11.561				
Paan, gutka and alcohol	35	P<0.01**				
Nil	64					
HLA A*3 Positive patients (N=108)						
First child	30	X^2_1=21.333				
Not first child	78	P<0.001***				
Taken together N=136						
First child	32	X^2_1=38.118				
No first child	104	P<0.001***				

*=p<0.05, **=p<0.01,***p=<0.001
* = significant

Over 40 studies have shown that the individuals who later develop schizophrenia have a 5 to 15% excess of winter and spring births (Boyd et al., 1986;Bradbury and Miller, 1985). Thus in our study we analyzed the seasonal birth pattern in schizophrenic patients. 62 patients for whom authentic date of birth were available have been included in this study and compared with 100 controls (Table 26). For this study we have divided the season of birth of the patients and controls into three categories, winter (Nov-Feb), summer (March-June) and monsoon (July-Oct) (Fig.31). Interestingly, there was an increase incidence of winter birth among the patients but when compared and analyzed statistically with the normal control, it was not found to be significant.

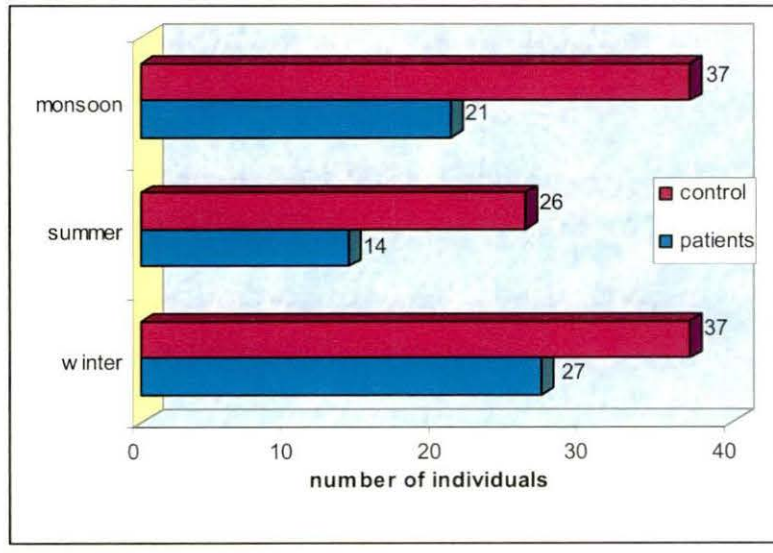

Figure 31: Showing bar diagram of season of birth among schizophrenic patients and normal individuals according to the season.

Again the birth pattern was studied according to months, which revealed the increase number of birth during February among the patients but still the finding was not significant (Fig.32). Thus our results do not corroborate with the winter birth hypothesis of schizophrenia. Even though the present study hinted the winter birth access in schizophrenics, small number of sample size greatly limited the study. More comprehensive study including large number of the samples may throw light in this respect.

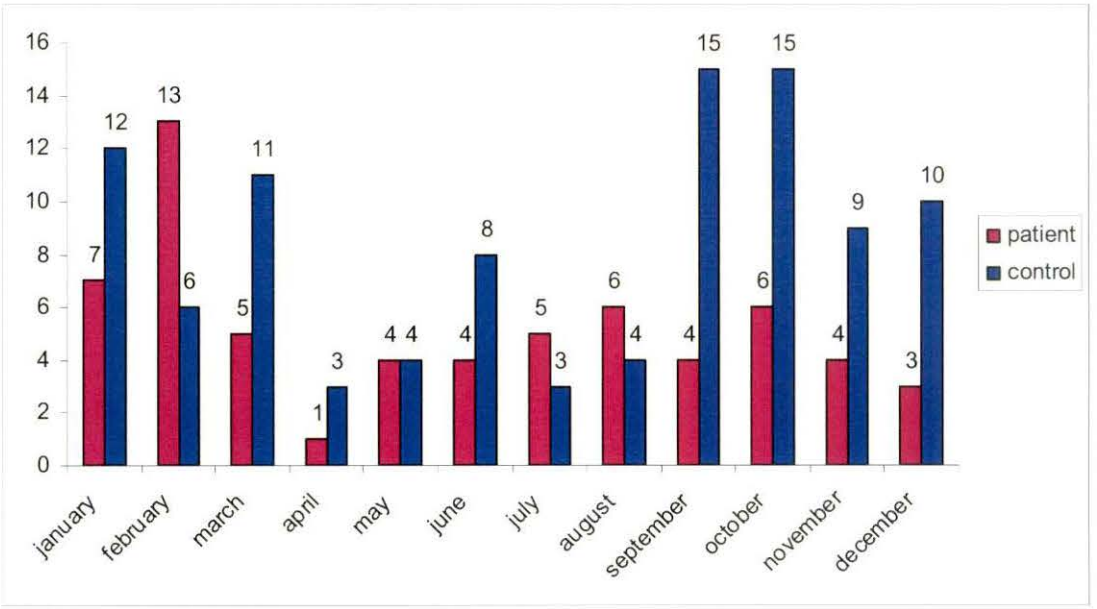

Figure 32: Showing bar diagram of season of birth among schizophrenic patients and normal individuals according to months.

Table 26: Season of birth among schizophrenic patients and normal individuals.

	Patients N=62		Control N=100		Chi square
Season of birth					
Winter (Nov-Feb)	27	$X^2_2 = 4.097$	37	$X^2_2=2.418$	$X^2_2=0.0701$
Summer (March-June)	14		26		
Monsoon (July-Oct)	21		37		
Season of birth					
Jan	7		12		
Feb	13		6		
March	5	$X^2_{10}=12.877$	11	$X^2_{10}=17.700$	$X^2_{10}=16.631$
April	1		3		
May	4		4		
June	4		8		
July	5		3		
Aug	6		4		
Sept	4		15		
Oct	6		15		
Nov	4		9		
Dec	3		10		

*=p<0.05, **=p<0.01,***p=<0.001
* = significant